14th Plant Development Workshop

You and your colleagues (graduate and undergraduate students and staff) are invited to attend the 14th Plant Development Workshop which will be held in the Botany Building at the University of Toronto on Saturday, November 3, 1984.

Preliminary schedule:

9:00 - 9:30 a.m.	Coffee and registration
9:30 - 12:00 noon	Contributed papers
12:00 - 2:00 p.m.	Lunch and poster session
2:00 - 4:00 p.m.	Contributed papers

Please use the attached form to send titles and abstracts for papers and posters by October 24, 1984 to:

Dr. Nancy G. Dengler

Department of Botany

University of Toronto

Toronto, Ontario M5S 1A1

(416)978-3536

NAME _____

NUMBER ATTENDING PLANT DEVELOPMENT WORKSHOP FROM YOUR LAB _____

TITLE
Author(s)
Abstract

TITLE
Author(s)
Abstract

botany

UNIVERSITY OF TORONTO · DEPARTMENT OF BOTANY · TORONTO, ONT. M5S 1A1

October 24, 1984

Dear Colleague,

This is a reminder of the 14th Plant Development Workshop to be held on Saturday, November 3, 1984 at the Department of Botany, University of Toronto. If you or others from your lab plan to attend, and I have not yet heard from you, please let me know by Wednesday, October 31, 1984.

For those of you planning to drive, a map showing parking areas is enclosed. Street parking on King's College Road and Circle and the lot "A" entered from Galbraith Road are closest to the Botany Building. (Parking is free on Saturday.) The Botany Building "B" is on the northwest corner of College Street and University Avenue at the Queen's Park subway station.

I am looking forward to seeing you on November 3, 1984.

Yours sincerely,

Nancy Dengler

Nancy G. Dengler
Associate Professor
(416) 978-3536

NGD/js

Encl.

THE 14TH PLANT DEVELOPMENT WORKSHOP

Saturday, November 3, 1984

Department of Botany
University of Toronto

9:00 - 9:30	Registration and coffee, Room 207C (Contributed papers in Room 7)
9:30 - 9:50	Xylogenesis in suspension cultures of Zinnia elegans: A study using immunofluroescent techniques. Marcia Falconer and Robert Seagull, Department of Biology, Carleton University.
9:50 - 10:10	Effects of taxol on elongation on elongating vetch cells. C. Weerdenburg, R.W. Seagull and G. Setterfield, Department of Biology, Carleton University.
10:10 - 10:30	The effects of anti-auxins and auxin transport inhibitors on cytokinen-induced caulogenesis by lettuce cotyledons. P.R. Forbert, D.P. Webb, R. Lee, L. Fagervik and A. Webb, Department of Biology, Queen's University.
10:30 - 10:50	Adventitious shoot initiation on excised embryos of Pinus strobus. D.P. Webb, B. Flinn and P. De la Bastiide, Department of Biology, Queen's University.
10:50 - 11:10	Coffee, Room 207C
11:10 - 11:30	The anatomy of the root apex of Regnellidium diphyllum. Ann Eastman and R.L. Peterson, Department of Biology, University of Waterloo and Department of Botany, University of Guelph.
11:30 - 11:50	Early stages in root colonization by VAM fungi. M.C. Brundrett, Y. Piche and R.L. Peterson, Department of Biology, University of Waterloo and Department of Botany, University of Guelph.
11:50 - 12:10	Cycad root nodulation in vitro. D.P. Webb, Department of Biology, Queen's University.
12:10 - 12:30	A morphometric study of effective nodules induced by Rhizobium loti and Bradyrhizobium sp. (lotus) on Lotus pedunculatus . William Newcomb, Susan M. Wood, David B. Layzell and C.E. Pankhurst, Department of Biology, Queen's University.
12:30 - 1:30	Lunch, Room 207 A, B, C
1:30 - 1:50	Studies of mineral reserves in pea (Pisum sativum) cotyledons using low water content procedures. John N.A. Lott, David Goodchild and Stuart Craig, Department of Biology, McMaster University and Plant Industry, C.S.I.R.O., Canberra.

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1:50 - 2:10 Occurence of globoid crystals in cotyledonary protein bodies of Pisum sativum as influenced by experimentally induced changes in Mg, Ca, and K content of seeds. John N.A. Lott, Peter J. Randall, David Goodchild and Stuart Craig, Department of Biology, McMaster University and Plant Industry, C.S.I.R.O., Canberra.

2:10 - 2:30 A new technique using disodium fluorescein to investigate symplastic connections between plant cells. Jacqueline A. Prazak and Carole A. Peterson, Department of Biology, University of Waterloo.

2:30 - 2:50 Relationship between structure and function of exodermis of many monocot and dicot roots. C.J. Perumalla, Daryl E. Enstone and Carol A. Peterson, Department of Biology, University of Waterloo.

2:50 - 3:10 Coffee, Room 207C

3:10 - 3:30 Developmental and genetic studies of floral trimorphism. S.C.H. Barrett, J.H. Richards and P.M. Donnolly, Department of Botany, University of Toronto and Department of Biological Sciences, Florida International University.

3:30 - 3:50 In vitro selection for animo acid overproduction in barley. M.S. Kuruvinashetti and K.J. Kasha, Department of Crop Science, University of Guelph.

3:50 - 4:10 Temperate woody species as interior landscape plants. R.A. Friendship, M.J. Tsujita and D.P. Ormrod, Department of Horticultural Science, University of Guelph.

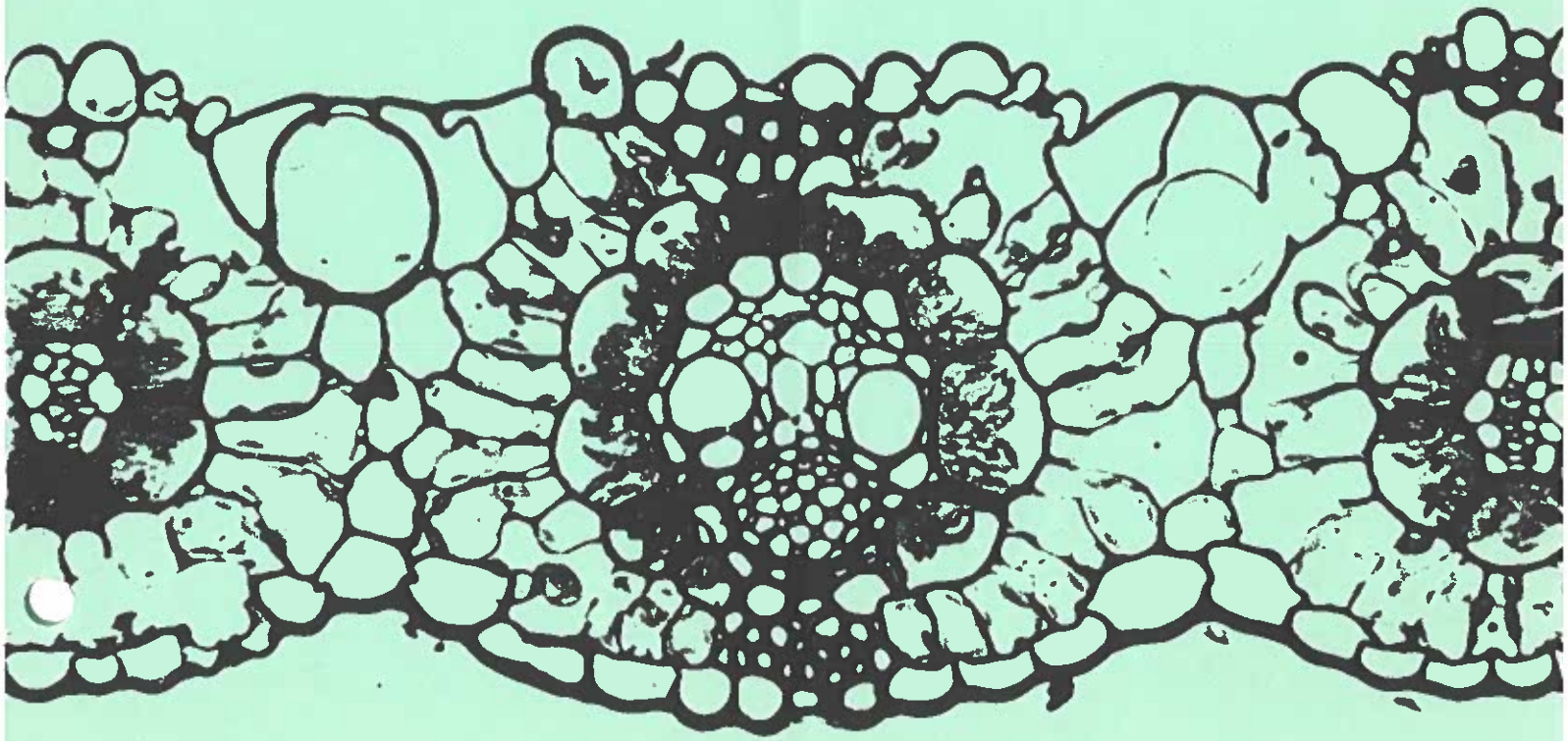
R. Douglas

14th Plant Development Workshop

Saturday, November 3, 1984

Department of Botany

University of Toronto



9:00 Coffee, registration. Room 207C

9:30 Contributed papers in Room 7

Xylogenesis in suspension cultures of Zinnia elegans: A study using immunofluorescent techniques. Marcia Falconer and Robert Seagull, Dept. of Biology, Carleton University, Ottawa.

Xylogenesis has been studied in primary suspension cultures derived from leaf mesophyll of Zinnia elegans L. The wall patterns produced in culture closely resemble those described for intact tissues (annular, spiral, sclalriform, reticulate, pitted). Using fluorescence microscopy and immunocytochemical techniques we have followed both the changes in microtubule organization and wall deposition during xylogenesis. Calcofluor White has been used to detect secondary wall deposition before it can be observed using either phase contrast or polarization optics. The development of tracheray elements can be divided into three stages: 1) microtubules grouped into bands without secondary wall deposition evident; 2) groups of microtubules subtending wall material only visible using Calcofluor White; 3) a complex microtubule pattern reflected by well developed wall thickenings detected using Calcofluor, phase contrast and polarization optics.

Cells of young cultures (3 days post inoculation) contain microtubules oriented parallel to the long axis. After several days, microtubule orientation shifts through 90 degrees to form parallel arrays which are perpendicular to the long axis of the cell. Only after this shift do cells undergo xylogenesis.

9:50 Effects of taxol on elongating vetch cells. C. Weerdenburg, R.W. Seagull and G. Setterfield, Dept. of Biology, Carleton University, Ottawa.

Suspension cultures of vetch (Vicia hajastana) cells were grown in medium supplemented with gibberellic acid for 48 h to induce cell elongation. Microtubules (MT) and newly deposited cellulose microfibrils in elongating cells were arranged parallel to each other in ordered arrays perpendicular to the long axis of the cell. Taxol, an alkaloid which both stabilizes MT and promotes MT polymerization in animal cells, was added to elongating vetch cells. After 72 h in taxol many cells were spheroid while others showed a central spherical swelling but retained cylindrical ends. Immunofluorescence microscopy using monoclonal anti-tubulin revealed that in the spheroidal regions bundles of MT were oriented tangentially and/or parallel to the long axis of the cell. Different orientations of MT were often present side by side in the same cell. In the cylindrical ends of partially swollen cells regular arrays of MT perpendicular to the long axis remained present. Electron microscopy of the inner surfaces of wall fragments from taxol treated cells showed adjacent bundles of parallel microfibrils oriented longitudinally and tangentially. Taxol apparently disrupts mechanisms which control overall coordination of MT orientation leading indirectly to partial disruption of cellulose organization and cell form.

10:10 EFFECTS OF ANTIAUXINS AND AUXIN TRANSPORT INHIBITORS ON CYTOKININ-INDUCED CAULOGENESIS FROM LETTUCE COTYLEDONS.
P. R. Fobert, D. T. Webb, R. Lee, L. Fagervik & A. D. Webb

Caulogenesis from cultured lettuce cotyledons can be induced by cytokinins in the absence of exogenous auxin. However, caulogenesis is stimulated by the addition of IAA. Since it is generally accepted that in vitro organogenesis is controlled by auxin and cytokinin at the sites of organ induction, we hypothesize that endogenous auxin must be present within the explant during cytokinin-induced shoot initiation. We investigated the possible role of endogenous auxin in cytokinin-induced caulogenesis by the application of the antiauxins 2,4,6-T & PCIB or auxin transport inhibitors TIBA & NPA with kinetin. All compounds tested inhibited shoot formation in the range of 100 uM. However, lower concentrations of TIBA (0.1 uM) and 2,4,6-T (1 uM) stimulated shoot production. Addition of IAA to media containing inhibitory levels of 2,4,6-T only partially overcame the inhibition of K-induced caulogenesis.

10:30 ADVENTITOUS SHOOT INDUCTION ON EXCISED EMBRYOS OF PINUS STROBUS. B. Flinn, D. T. Webb and P. de la Bastide.

On SH medium, adventitous shoot initiation by excised embryos of Pinus strobus (Eastern White Pine) requires a cytokinin, but does not require nor is it enhanced by seed pretreatments at 5 or 27 C. Both BA and 2iP induced shoot formation over a wide range of concentrations, but BA had a much lower optimal concentration. Shoots developed primarily from cotyledonary callus. Undifferentiated callus formation was also observed, especially at supra-optimal BA concentrations. The duration of exposure to cytokinin (2,4,6,8 weeks) did not significantly affect the average number of shoots formed. However, longer exposures to cytokinin caused increased callus formation.

10:50 Coffee, Room 207C

11:10 THE ANATOMY OF THE ROOT APEX OF REGNELLIDIUM DIPHYLLUM

Ann Eastman and R.L. Peterson

The root apex of Regnellidium diphyllum consists of a tetrahedral apical cell that forms, proximally, three sectors or merophytes. Each merophyte gives rise to cells of the epidermis, cortex and stele and, distally, a root cap initial. Numerous plasmodesmata traverse the walls of the apical cell and proximal derivatives. Based on position and cytological features, the root cap of seven or eight tiers of cells consists of columella and peripheral regions. Columella cells are distinguished by the presence of numerous amyloplasts with large starch grains whereas peripheral cells contain smaller, degenerating amyloplasts, have thickened periclinal walls and secrete mucilage. Histochemical procedures show that the mucilage consists of proteins and carbohydrates. Older peripheral cells have phenolic deposits in the vacuoles and aniline blue- positive substances in the cell walls. The root cap sloughs intact tiers of cells unlike most root caps which

11:30 Microsporogenesis in Pinus banksiana Lamb: A histochemical study using resin-embedded sections fixed in glutaraldehyde. Mary I. Moore, Box 159, Deep River, Ontario

A histochemical study of the microsporangium of Pinus banksiana Lamb. from initiation to pollen maturation and release is reported. Differentiation of the tapetum is shown to be from sporogenous cells. The apparent flow of large ammounts of fluid into the sporangium is associated with changes in microsporocytes and in the tapetum. Pollen mother cells are followed through meiosis, the tetrad period, and release of microspores into thecal fluid when changes, as determined by staining techniques occur in the outer pollen wall and the orbicules. A callose-fluorescing intine is present. The uninucleate microspores develop into mature pollen grains as the tapetum dissolves and sporopollenin is released.

11:50 CYCAD ROOT NODULATION IN VITRO.
D. T. WEBB

Seedlings of most cycads form a tap root system with typical plagiotropic laterals when grown on mineral medium plus 2% sucrose in darkness. However, with Macrozamia communis, apogeotropic root nodules develop at the root-shoot junction in the dark, but more acropetally formed roots have pointed apices and are plagiotropic. Apogeotropic roots also develop at the root-shoot junction of Encephalartos seedlings, but these are not nodular. With all species except Dioon edule, light induces nodulation of plagiotropic laterals. These nodules branch dichotomously, lack a distinct root cap, have a broad apical meristem, exhibit superficial cell hypertrophy, and form a presumptive algal zone. With M. communis, the stele of nodules and lateral roots is diarch with a distinct endodermis. Xylem development is principally exarch with some mesarch differentiation. There is more xylem development in root nodules than in plagiotropic laterals, but both types of roots are very similar in their vasculature. Preliminary work with Zamia pumila indicates that NAA can partialy substitute for light as a stimulus for nodulation.

12:10 A MORPHOMETRIC STUDY OF EFFECTIVE NODULES INDUCED BY RHIZOBIUM LOTI AND BRADYRHIZOBIUM SP. (LOTUS) ON LOTUS PEDUNCULATUS William Newcomb, Susan M. Wood, David B. Layzell and C.E. Pankhurst, Dept. of Biology, Queen's University, Kingston, Ontario K7L 3N6.

Effective root nodules formed on Lotus pedunculatus by Rhizobium loti NZP2037 and Bradyrhizobium sp. (Lotus) CC814s were examined by LM and TEM. NZP2037 nodules were larger than CC814s nodules as a result of a much thicker nodule cortex. The smaller CC814s nodules had a much larger infected zone and consequently, larger volumes of infected cells, peribacteroid space and host cytosol per nodule. CC814s nodules had a significantly higher number of bacteria which typically contained prominent polar bodies and beta-polyhydroxybutyrate (PHB) granules. The bacteria of NZP2037 nodules lacked the polar bodies but PHB deposits were occasionally observed.

The acetylene reduction assay showed CC814s nodules to be more active than NZP2037 nodules. This measured difference in nitrogen-fixing activity was considered due to the degree of infection and bacterial proliferation as

12:30 Lunch, Room 207C

1:30 Contributed papers, Room 7.

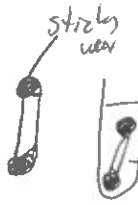
Studies of Mineral Reserves in Pea (Pisum sativum) Cotyledons using Low Water Content Procedures by John N.A. Lott[1], David J. Goodchild[2], and Stuart Craig[2]
1. Biology Department, McMaster University and
2. CSIRO, Division of Plant Industry, Canberra, Australia
Most of the phytin in pea (Pisum sativum) cotyledons is water soluble. In order to determine where K and P are located it was necessary to use anhydrous or low water content tissue preparation procedures to obtain samples suitable for energy dispersive x-ray analysis studies using a transmission electron microscope. While some protein bodies do contain electron-dense globoid crystals most do not. Globoid crystals are more prevalent in the abaxial part of the cotyledon where the provascular network is located. When present, globoid crystals contain considerable Mg and/or Ca along with P and K. Protein bodies that lack globoid crystals still contain considerable P and K with lesser amounts of elements such as S, Cℓ and Mg. This is consistent with these protein bodies containing a considerable amount of K-phytate in the proteinaceous matrix. While there is a lot of K inside the protein bodies, K is widespread in pea cotyledon tissue and could be detected in starch grains, cell walls and the cytoplasmic matrix.

1:50 Occurrence of Globoid Crystals in Cotyledonary Protein Bodies of Pisum sativum as Influenced by Experimentally Induced Changes in Mg, Ca and K Contents in Seeds.
John N.A. Lott[1], Peter J. Randall[2], David J. Goodchild[2] and Stuart Craig[2]
1. Biology Department, McMaster University and
2. CSIRO, Division of Plant Industry, Canberra, Australia.
In many species globoid crystals in protein bodies of seeds are very common while in other species they are rarely observed. A review of literature suggested that the balance between divalent (Mg^{++} and Ca^{++}) and monovalent (K^{+}) cations may be important in determining whether or not globoid crystals will form. To test this hypothesis experiments were carried out to add Mg and Ca, or Ca alone, to pods developing on K-deficient pea plants. While it was possible to cause a reduction in K concentration and increases in Mg and Ca concentrations, any changes to the normal mineral storage pattern in pea cotyledons were remarkably small. In some treatments statistically significant increases in the ration (Mg + Ca)/K were obtained and the sample with the greatest increase was examined in detail by electron microscopy and energy dispersive X-ray analysis. In this sample globoid crystals were common in contrast to their normally rare occurrence in pea cotyledonary tissue.

2:10

A NEW TECHNIQUE USING DISODIUM FLUORESCEIN TO INVESTIGATE SYMPLASTIC CONNECTIONS BETWEEN PLANT CELLS

Jacqueline A. Prazak and Carole A. Peterson



- thicker xs + viewed w/ UV
- older tissue posed problem
- must use thick sections for intact cells

There is controversy as to whether the mechanism of sucrose movement from the vascular tissue to the cortex is symplastic or apoplastic in roots. This prompted investigation using a symplastically mobile dye disodium fluorescein, to follow symplastic continuity between the pericycle and the vascular cylinder. Guard cells have been determined by electron microscopy to be symplastically isolated from other epidermal cells. Thus, Tradescantia sp. epidermis was used to test the suitability of fluorescein to indicate symplastic isolation in living tissue. As the technique seemed valid, excised, sealed segments of 3-day old Zea mays roots were incubated in a fluorescein solution for 6 H and free-hand transverse sections were viewed with ultra-violet light. The fluorescein entered the root as far as the pericycle and was not apparent in the vascular tissue. This technique is potentially more accurate and rapid for determining symplastic continuity than the routinely used methods of electron microscopy.

2:30

Relationship between structure and function of exodermis of many monocot and dicot roots.

C.J. Perumalla, Daryl E. Enstone and Carol A. Peterson

The chemical composition and structure of certain cell types is known to play a very important physiological role. The suberized and sometimes lignified Casparian band in the radial walls of the endodermis of most plants and exodermis of corn and onion form an apoplastic barrier to water and ions entering into the stele and the cortex of the root. A variety of tests were conducted on fifty species to detect the presence of a Casparian band in the exodermis. Studies included viewing uncleared autofluorescing cross sections and cleared sections. Permeability studies were conducted using the apoplastic fluorescent dye, Calcofluor white M2R (CFW). The radial walls of exodermis of all but two species were impermeable to the dye. However, after wounding CFW passed through the break, in the exodermis and penetrated several cortical layers on the basis of the above studies and histochemical tests. It is concluded that a Casparian band exists in the exodermis of single and multilayered types of all but two of the species studied.

- Calcofluor found appropriate dye
- Casp. strips acid dig. resistant (exodermis, endodermis + lignified tracheary elements, in above acid not dig.)
- hand sections fluorescence test
- sudan, sudan + phloroglucinol (sometimes) - show casp. strip

2:50 Coffee, Room 207C

3:10 Developmental nad Genetic Studies of Floral Trimorphism:
S.C.H. Barrett, J.H. Richards and P.M. Donnelly

Tristyly is a genetic polymorphism in which plant populations are composed of three floral morphs that differ from one another in style length, anther height, pollen size and incompatibility behaviour. In Eichhornia paniculata, a diploid, short-lived perennial the polymorphism is controlled by two linked loci (S, M) with two alleles at each locus and S epistatic to M. Flowers of each morph have two whorls of three tepals, six stamens and three fused carpels. The six stamens differentiate into two sets of three stamens each but stamens from both whorls make up a given set. Floral differences among morphs are not present at flower origin or floral organ initiation and arise first among stamen sets. The two sets within a flower differ prior to meiosis in the size, number and timing of comparable developmental events in the sporogenous cells. Later anther size diverges and differences in filament and floral tube length, cell size, cell number as well as differences in the length, cell size, and cell number of styles develop among the morphs. Tristyly in E. paniculata has broken down to give rise to self-pollinating semi-homostylous mid-styled forms. Modifications of the mid morph involve increase in filament length of short-level stamens. Selfing genotypes exhibit a high degree developmental instability of floral traits.

3:30 In Vitro Selection for Amino Acid Overproduction in Barley

M.S. Kuruvinashetti and K.J. Kasha
Department of Crop Science, University of Guelph

Excised M2 embryos, callus and suspension cultures were screened against inhibitory levels of lysine plus threonine (LT) and S-2-aminoethyl cysteine (AEC). Several variants were isolated based on their ability to grow on these selective media. Among many escapes, some lines that inherited the selected trait in subsequent generations have been identified. Most selections were made on young calli that were in the process of organogenesis to avoid the problem of loss of regenerative potential in older cultures. It seems that in segregating generations, the homozygous condition for LT resistance can invariably be identified based on the colour of the scutellum on selective medium. However, LT resistance itself appears to be inherited as a dominant character. A couple of variants isolated for AEC tolerance are marginally better in growth than controls on AEC medium. Further studies to characterize these lines with respect to seed protein and amino acid profiles are under way.

3:50

Temperate woody species as interior landscape plants.
R.A. Friendship, M.J. Tsujita and D.P. Ormrod, University of Guelph.

The feasibility of employing temperate woody species in the interior landscape was examined. The indoor performance of 19 deciduous and evergreen species was observed. Taxus, Caragana, Cornus, Ilex, Acer and Euonymus showed potential for indoor use. Condition of plants at the time of introduction to the interior environment was also investigated, including light acclimation of the material before placement. Installation of dormant splants appeared to be more successful than the installation of actively

9:30 - 4:30 Poster Session, Room 207B

Early Stages in Root Colonization by Vam Fungi

M. C. Brundrett, Y. Piche and R. L. Peterson

A new experimental procedure was developed to produce samples of leek roots containing early stages in colonization by vesicular-arbuscular mycorrhizal (VAM) fungi. This procedure involved transplanting leek seedlings into a pot culture containing an established symbiosis. Abundant colonization of seedling roots occurred in one week. Samples harvested in two day intervals after exposure to the inoculum were used to determine the time-course of colonization. The average distance from growing root apices at which each stage in root colonization first occurred and the growth rate of intercellular hyphae were also estimated. These stages were then documented at the light microscope level. This procedure should allow the production of abundant material at early stages in VAM development for chemical and morphological investigations as well as providing an efficient inoculation procedure.

Mid April '85 - Hort. Sci.

Late April, ecos thing?